DNA isolation from yeast

Procedure

1. Grow yeast ON and transfer 1.5 ml (consider the cell density/flocculating to make sure to have enough cells) of the culture to an eppendorf tube.
2. Spin down the cells for 2 min at 5000g, then resuspend in 0.6 ml Lysis buffer.
3. Transfer solution to a screw cap and add glass beads to approximately 1/3-1/2 of the height of the tube,
4. Add 0.6 ml Phenol/chloroform (1:1) to the mixture and then close the tube by tightly fastening the cap. Work with phenol and chloroform in the fume hood!
5. Disrupt cell walls by shaking in bead-beater at macumym setting for 2*1 min, keep on ice in between. Then transfer the aqueous (upper) layer to a new eppendorf tube.
6. Extract traces if remaining phenol by adding 200 µl chloroform. Vortex. Centrifuge at 13000g for 1 min and transfer the aqueous layer to a fresh eppendorf tube.
7. Add 1 volume of RT isopropanol to precipitate the DNA. Centrifuge (+4°C) at 16000g for 20 minutes. Carefully decant supernatant without disrupting the cell pellet (should be clearly visible).
8. Wash pellet with 1 ml 70% ethanol. Centrifuge (+4°C) at 16000g for 5 minutes. Decant supernatant and dry off remaining liquid.
9. Resuspend DNA in 100 µl TE. Measure DNA concentration at 260 nm.

Lysis buffer:

1% SDS
100 mM NaCl
2% TritonX-100
1x TE (10 mM Tris-Cl, 1 mM EDTA, pH 8)